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Studies on Acid Deoxyribonuclease

VII. Conformation of Three Nucleases in Solution[1, 2]

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The optical rotatory dispersion, circular dichroism, and infrared spectra of acid DNase, pancreatic DNase, and *Staphylococcus aureus* nuclease have been determined. The conformations of these three nucleases differ greatly from each other and from that of ribonuclease; while the acid and pancreatic DNases contain little α-helix, the bacterial enzyme has *ca.* 25% of that conformation; acid DNase probably contains antiparallel pleated sheet β-structure.

Spleen acid deoxyribonuclease (DNase), an enzyme isolated as a homogeneous protein (1, 2) and characterized in several of its physical and chemical properties (3), has been the object of extensive investigations in recent years. These have been reviewed elsewhere (4, 5).

Two prominent features of spleen acid DNase are its ability to split simultaneously both strands of native DNA at the same level (6) and its dimeric structure (7). It is evident at this point that structural investigations on the enzyme could be extremely rewarding. As a first step in this direction, we decided to undertake a conformational study on acid DNase and to compare the results obtained with similar data obtained on two related enzymes, namely, pancreatic DNase and the nuclease isolated from *Staphylococcus aureus* (*S. aureus* nuclease). A preliminary report has been presented elsewhere (8).

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MATERIALS AND METHODS

Materials. Acid DNase B was prepared from hog spleen by the method of Bernardi *et al.* (2). Preparation HS 21 was used in the present work. Pancreatic DNase (1 × crystallized) was purchased from Worthington Biochemical Corp. (D, lot No. 81A). The *S. aureus* nuclease was also a Worthington product (NFCP, lot No. 8FB, 11,000 units/mg).

Methods. The optical rotatory dispersion (ORD) and circular dichroism (CD) spectra were obtained on a Cary 60 recording spectropolarimeter equipped with a Model 6001 accessory, using cells with 1.0, 0.1, and 0.01 cm path lengths between 185 and 350 nm and protein concentrations of approximately 1 mg/ml. The visible range data for the Moffitt–Yang (9) and Schechter–Blout (10)

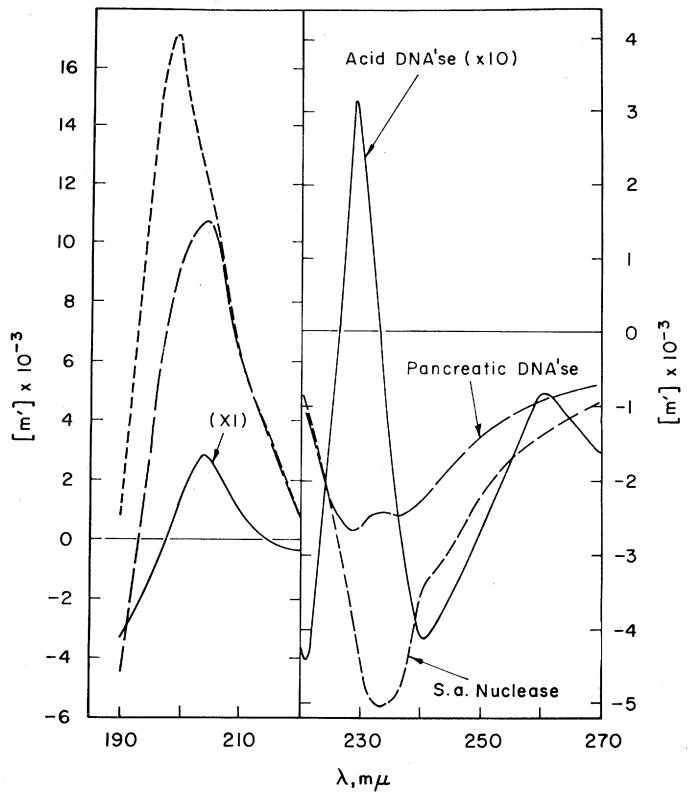

FIG. 1. Far-UV ORD spectra of three nucleases. Note: The spectrum of acid DNase above 220 nm is expanded tenfold.

plots were obtained in a 5-cm cell. All data were corrected for the refractive index of the solvent, sodium phosphate buffer, pH 7.0, $\Gamma/2 = 0.1$. The infrared spectra were measured between 1,400 and 1,800 cm^{-1} on a double beam grating Perkin Elmer Model 621 spectrophotometer in a phosphate buffer (pH 7.0, $\Gamma/2 = 0.1$) using a 0.1-mm path length cell with calcium fluoride windows; the protein concentration was *ca.* 10 g/l. for the acid and pancreatic DNases and 4 g/l. for *S. aureus* nuclease.

RESULTS AND DISCUSSION

ORD. The results of the ORD studies are presented in Fig. 1 and Table I. The data obtained in the visible region, shown in Table I, reveal strong differences between the conformations of the three enzymes. Both the Moffitt–Yang (9) a_0 and b_0 parameters and the Schechter–Blout (10) H_{193} and H_{225} parameters indicate that acid DNase has essentially no α-helix; b_0 is small and positive as is H_{193} ; H_{225} actually assumes a negative value. The lack of agreement between the H_{193} and H_{225} parameters and the concomitant small negative value of a_0 indicate strongly that the conformation of this enzyme consists of a mixture of unordered structure and some other, possibly ordered, conformation which is not an α-helix. The case is similar with pancreatic DNase; here, the small negative value of b_0 indicates the presence of not more than 15% α-helix, while the value of a_0 and the lack of agreement between H_{193} and H_{225}

TABLE I

OPTICAL ROTATORY DISPERSION OF THREE NUCLEASES

Enzyme	a_0	b_0	H_{193}	H_{225}
Acid DNase	−74	50	14.0	−8.6
Pancreatic DNase	−291	−40	20.9	10.4
S. aureus nuclease	−341	−182	36.0	32.9

points to a mixture of unordered and other (possibly β) conformations. In the case of the *S. aureus* nuclease, the situation is different; both b_0 and the Schechter–Blout parameters suggest the presence of 25–35% α-helix, in fair agreement with Heins *et al.* (11); the similarity in the values of H_{193} and H_{225} suggests that most of the remainder of the molecule is in unordered conformation.

The far-ultraviolet ORD results are shown in Fig. 1. The spectrum of acid DNase is quite complex. It is characterized by weak peaks at 229 nm ([m'] = 325) and 204 nm ([m'] = 2,840) and troughs at 240 nm ([m'] = −415) and 221 nm ([m'] = −450). While this spectrum defies analysis in terms of conformation, it is somewhat reminiscent of the spectra of some proteins that contain β-structure, such as γ-globulin (12, 13), which also have weak, complex far-ultraviolet ORD spectra. It is interesting to note, furthermore, that acid DNase has one of the weakest reported rotations at the sodium D line for a globular protein; $[\alpha]_D$ = −11. This reflects the unusually low intensities of the far-ultraviolet Cotton effects. The peak at 204 nm suggests the presence of β-conformation (14, 15). The details of the ORD pattern above 220 nm most probably reflect contributions of side chain chromophores. The ORD spectrum of pancreatic DNase is characterized by a bimodal trough with minima at 228 nm ([m′] = −2,680) and 236 nm ([m'] = −2,485) and a peak at 204 nm ([m'] = 10,800). This spectrum is very similar to that of β-lactoglobulin (16) which is believed to contain antiparallel -pleated sheet conformation (17), and might be taken as an indication of the presence of that conformation, in agreement with Cheng (18). The ORD spectrum of *S. aureus* nuclease again is consistent with the presence of a considerable amount of α-helix; it has a peak at 199 nm, a shoulder at 215 nm, and a

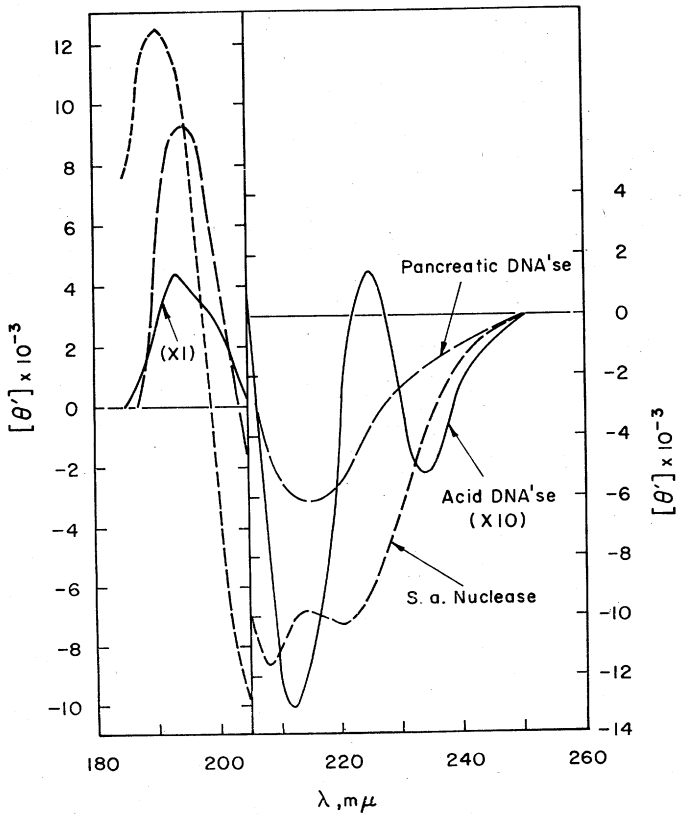

FIG. 2. Far-UV CD spectra of three nucleases. Note: The spectrum of acid DNase above 205 nm is expanded tenfold.

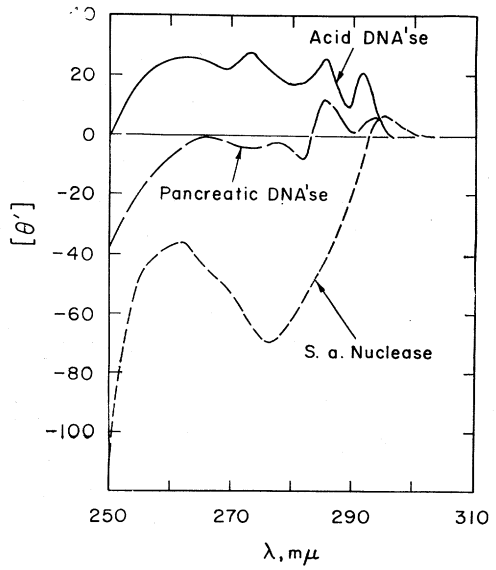

FIG. 3. Near-UV CD spectra of three nucleases.

trough at 233 nm. The negative shoulder at 245 nm may reflect the contribution of an aromatic chromophore.

CD. The CD spectra of the three enzymes are presented in Figs. 2 and 3. In the far-ultraviolet, the situation is again very similar to that found in ORD. Acid DNase has a weak complex spectrum. Negative maxima are found at 234 nm ($[\theta'] = -535$) and 212 nm ($[\theta'] = -1{,}320$), while positive maxima are observed at 225 nm ($[\theta'] = 145$ and 193 nm ($[\theta'] = 4{,}175$). The last band suggests again the presence of β-structure (19), while the great detail above 205 nm probably reflects the contribution of side chain chromophores. The spectrum of pancreatic DNase strongly suggests the presence of β-structure (positive peak at 194 nm, negative peak at 215 nm), while that of the *S. aureus* nuclease is in agreement with that reported above 210 nm (20) and is consistent with the presence of approximately 20–25% α-helix, a number in reasonable agreement with the recently reported X-ray crystallographic data (21).

The near-ultraviolet CD spectra of the three enzymes are shown in Fig. 3. Both acid DNase and pancreatic DNase display very weak and complicated CD patterns. Both have positive maxima at 292–293 nm and at 285 nm which probably reflect tryptophan transitions (22, 23) [acid DNase has 6 Trp (3), the pancreatic enzyme has 4 Trp (24)]; the rest of the spectrum may reflect overlapping transitions of the tyrosine side chains and the disulfide bridges (25, 26) [acid DNase has 12 Tyr and 4 S-S (3), pancreatic DNase has 16 Tyr and 2 S-S (24)]. The spectrum of the *S. aureus* nuclease is much simpler; it is dominated by a negative band at 276 nm and a weak positive maximum at 295 nm. In view of the absence of disulfide bridges in this protein (27), the 276-nm band may be assigned to the 7 tyrosines, while the positive absorption at 295 nm probably reflects transitions of the single tryptophan (22, 23). In general, with the exception of the positive absorption at 295 nm, the CD spectrum of the bacterial enzyme is highly reminiscent of that of ribonuclease above 260 nm (25, 28, 29).

Infrared. The infrared spectra of the three enzymes are shown in Fig. 4. Restricting the discussion to the amide I band, which is

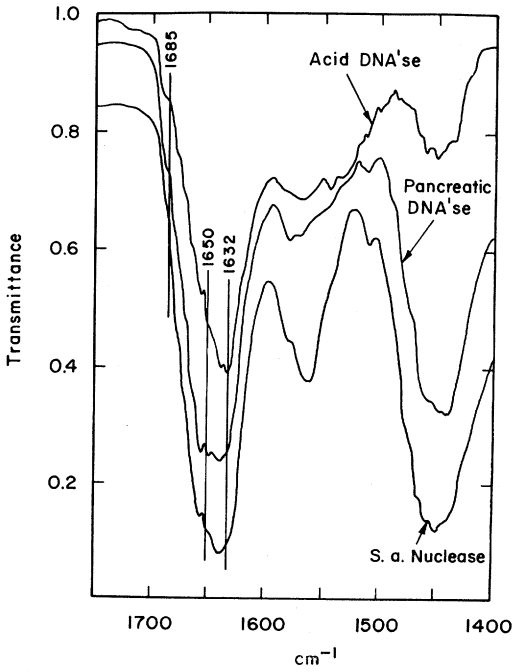

FIG. 4. Infrared spectra of three nucleases. The spectra of pancreatic DNase and *S. aureus* nuclease are arbitrarily displaced from 100% transmittance at 1,750 cm^{-1}; the spectrum of *S. aureus* nuclease was recorded with a scale expansion factor of 2.

dominated by carbonyl stretching (30), it can be seen that the acid DNase band is maximal at 1,632 cm^{-1} which is characteristic of β-structured proteins in solution (31). The shoulder at 1,685 cm^{-1} indicates that this β-structure is of an antiparallel pleated sheet type (32). The spectra of the other two enzymes are less definite. Both are characterized by rather broad amide I bands, maximal at *ca.* 1,640 cm^{-1}; the strong absorbance at 1,650 cm^{-1} is consistent with the presence of some α-helix, while the shoulders at 1,630 cm^{-1} could reflect some β-structure (30–32); these last two spectra, however, do not make possible an unequivocal assignment of structure.

CONCLUSIONS

Comparison of the ORD, CD, and infrared spectra of three functionally related enzymes reveals that their secondary conformations are greatly different from each other. Furthermore, it appears that their conformations are also different from that of another related enzyme, namely, pancreatic ribonuclease. The most likely conclusion on their conformations is that *S. aureus* nuclease contains in solution *ca.* 20–30% α-helix, with the remainder mostly unordered, in agreement with the crystal structure data (21); acid and pancreatic DNase both contain little or no α-helix, their secondary structures being predominantly in the unordered and β-conformations; acid DNase probably contains significant amounts of antiparallel pleated sheet structure, as shown by its infrared spectrum.

The ORD and CD spectra of acid DNase may serve, furthermore, as good example of the magnitude of the contributions that may be made below 250 nm by side chain transition; the weak complicated spectra are most probably reflections of transitions due to side chains rather than to the backbone polypeptide chain. Furthermore, the assignment of ORD and CD spectra in the far ultraviolet to β- and unordered conformations is quite hazardous. Fasman and Potter (33) have shown that polypeptides in the antiparallel pleated sheet β-conformation may have highly different spectra, band positions being displaced by as much as 10 nm depending on whether the structure belongs to the I-β or II-β family of spectra (22, 33). In the case of unordered conformation, polypeptides in solution give a CD spectrum with a deep negative band at 195 nm, weak positive absorption at 218 nm, and a very weak negative band at 235 nm (14). The same polypeptides cast as films give spectra with a negative band at 200–205 nm and a negative shoulder at 215–225 nm, a situation very similar to that observed with denatured proteins (34).

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